Revised sampling scheme for LBNL locations – Sept 20, 2010

Objectives this is meant to address:

1) duplication at a site (having two cores from each location to provide actual replicates, as opposed to pseudo-replicates from a single core)
2) cold-preserved material that would allow culturing, RNAlater-preserved material for transcriptomics, and glycerol-preserved material for single-cell genomics.

Option 1 (most preferred) – This option requires two intact cores from a drop and access to a third core that was used by another group that collected sub-cores inside an 11-cm core. This option provides two complete replicates and material for additional analyses, as detailed below. If this is not possible, please see Option 2 instead.

Procedure

Sediment processing:

Collect 2 cores from a drop and process water as described below. Freeze both cores at -80°C. This will allow us to have duplicates for each analysis. From a third core, collect 0.5 g sediment for single-cell genomics (store in glycerol at -80°C), 1 mL of sediment from the 0-1 cm range for transcriptomics (store at -80°C in RNA*later*), 1 mL of sediment for storage at 4°C (for culture work), and one mL (or 0.5 g) sediment for preservation in formaldehyde and then 4°C. The 0.5 g sediment could be collected using a cut-off 1-mL tip, whereas the 1-mL sediment could be collected using a cut-off 10-mL syringe (inserted into the sediment to 1 cm depth).

Overlying Water processing:

From one of the two cores that will be frozen, the water will have two things done. First, 18 mL will be preserved for AODC. Second, the remaining water that can be collected will be filtered and the filter will be stored at -80°C.

From the other core that will be frozen, the water will have four things done. First, 1.5 mL will be transferred to a tube with 0.5 mL 60% glycerol and stored at -80°C for single-cell genomics. If there is enough water to get three tubes for single-cell genomics, please do that. 18 mL will be preserved for AODC (so, transfer to a tube with 2 mL formaldehyde as usual). 10 – 20 mL of water will be stored in a tube at 4°C for culture work. The remaining water will be filtered, and the filter will be stored in RNA*later* at -80°C.

Option 2 (next preferred) – This option requires two cores total from one drop. It provides for partial duplication at a location.

Sediment processing:

Collect 2 cores from a drop and process water as described below. Freeze one core at -80°C without any sub-sampling. From the second core, collect 0.5 g sediment for

single-cell genomics (store in glycerol at -80°C), 1 mL of sediment from the 0-1 cm range for transcriptomics (store at -80°C in RNA*later*), 1 mL of sediment for storage at 4°C (for culture work), and one mL (or 0.5 g) sediment for preservation in formaldehyde and then 4°C. The 0.5 g sediment could be collected using a cut-off 1-mL tip, whereas the 1-mL sediment could be collected using a cut-off 10-mL syringe (inserted into the sediment to 1 cm depth). Store the remaining core at -80°C. We will process it as best we can for any 'duplicate' analyses once at LBNL.

Overlying Water processing:
From one of the two cores, the water will have two things done. First, 18 mL will be preserved for AODC. Second, the remaining water that can be collected will be filtered and the filter will be stored at -80°C.

From the other core, the water will have four things done. First, 1.5 mL will be transferred to a tube with 0.5 mL 60% glycerol and stored at -80°C for single-cell genomics. If there is enough water to get three tubes for single-cell genomics, please do that. 18 mL will be preserved for AODC (so, transfer to a tube with 2 mL formaldehyde as usual). 10 – 20 mL of water will be stored in a tube at 4°C for culture work. The remaining water will be filtered, and the filter will be stored in RNA*later* at -80°C.

Option 3 – This option only uses one core per location. It does not allow for any duplication, but will provide material for single-cell genomics, RNA work, and culturing from our key locations. This option also means there will be PLFA analysis available only from the sediment (not from any overlying water).

Sediment processing:
Collect 1 core from a drop and process water as described below. Freeze the core at -80°C without any sub-sampling.

Overlying Water processing:
The water will have four things done. First, 1.5 mL will be transferred to a tube with 0.5 mL 60% glycerol and stored at -80°C for single-cell genomics. If there is enough water to get three tubes for single-cell genomics, please do that. 18 mL will be preserved for AODC (so, transfer to a tube with 2 mL formaldehyde as usual). 10 – 20 mL of water will be stored in a tube at 4°C for culture work. The remaining water will be filtered, and the filter will be stored in RNA*later* at -80°C.

** While it is stated that this is only planned for the LBNL locations, please note that if any of the other locations prove to have oil one the sediment surface, it would be great to have the same thing done for those locations (at least Option 2 if at all possible). **